Modulation of SARS-CoV-2 Spike induced-UPR in HEK 293 T cells by selected small molecule inhibitors

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Introduction

- Coronavirus disease 2019 (COVID-19) is a potentially lethal, debilitating infectious disease caused by SARS-CoV-2.
- Coronavirus infection is initiated by the binding of the viral Spike (S) protein to the angiotensin-converting enzyme 2 (ACE2) cell surface receptors followed by fusion of the virus and host cell membrane to release the viral genome into the cell. Once inside, Coronaviruses (CoV) exploits the endoplasmic reticulum (ER) of the host cells for replication and in doing so, increases ER stress, evokes Unfolded Protein Response (UPR) and possibly autophagy, which could all attribute to the pathophysiology of the viral infections.
- To date, little is known about the roles of ER stress, UPR, and autophagy in SARS-CoV-2 infection. Here we over-expressed the viral Spike (S) protein in cultured HEK293T cells, as it has been shown that such protein is largely responsible for UPR activation in other CoV-infected cells.

Results

(1) UPR is activated upon SARS-CoV-2 Spike protein overexpression in cultured cells

In HEK293T cells over-expressing the SARS-CoV-2 Spike protein, the level of BIP/GRP78 and phosphorylated eIF2α were significantly higher compared to the transfected control.

(2) SARS-CoV-2 Spike protein expression triggers autophagy response in cultured cells

The involvement of autophagy in cells over-expressing the SARS-CoV-2 Spike protein were evaluated by monitoring levels of autophagy markers, LC3-I and LC3-II 24 hours after transfection.

(3) UPR modulators reduced the UPR and autophagy in cultured cells

HEK293T cells were treated with 4-PBA, TUDCA, DBM, Trazadone, and Salubrinal at indicated concentrations 4 hours after plasmid encoding Spike protein transfection.

Figure 1. Upregulation of BIP/GRP78 in HEK293T cells over-expressing the SARS-CoV-2 Spike protein. HEK293T cells at a density of 4×10^4 cells per well were transfected with 5 μg pcDNA3.1-SARS2-Spike and the protein levels of (a) BIP/GRP78 and (b) phosphorylated eIF2α (p-eIF2α) were determined by Western blotting using specific antibodies 24 hours after transfection. Quantified values of the specific protein abundance, which were normalized to the abundance of GAPDH, were included at the right panel.

Figure 2. SARS-CoV-2 Spike protein over-expression triggers autophagy through UPR. (a) HEK293T cells were transfected with 5μg pcDNA3.1-SARS2-Spike and the protein levels LC3II and LC3-I were determined by Western blotting using anti-LC3B antibody after 24 hours. Values were normalized to GAPDH. The ratio of LC3-II over GAPDH is graphically represented on right. (b) Cell viability of the transfected cells was evaluated by MTT assay, and the quantified values were normalized to non-transfected controls.

Figure 3. UPR modulator treatment reduced levels of ER stress, UPR and autophagy. 4 hours after transfection, the cells were treated with the selected UPR modulators, Salubrinal (50μM), 4-PBA (2mM), TUDCA (100μM), DBM (50μM) and Trazadone (50μM) for 24 hours. The protein levels of (a) BIP/GRP78, (b) phosphorylated eIF2α were determined by Western blotting using specific antibodies. Values were normalized to the abundance of GAPDH and were presented in the graphs. c) The protein levels of LC3-II with UPR modulator treatment were determined by Western blotting and normalized to the abundance of GAPDH. The ratio of LC3 II over GAPDH is graphically represented on right.

Conclusion

Based on the results above, we propose the following model to illustrate the interplay between SARS-CoV-2 infection and the ER stress pathway:

Figure 4. Model depicting PERK-eIF2α mediated autophagy activation by SARS-CoV-2 infection. SARS-CoV-2 infection induces ER stress by activating PERK arm of UPR. Phosphorylation of eIF2α by PERK attenuates global protein synthesis and leads to activation of autophagy. UPR modulators such as PBA, TUDCA, DBM, Trazadone and Salubrinal reduces the BIP and PBA and TUDCA reduces the autophagy by modulating PERK arm of UPR.